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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

DYMECKI

Appln. No. 08/866,279 — Group Art Unit: 1632

Filed: May 30, 1997 — Examiner: A.-M. Baker

FOR: USE OF FUNCTIONAL RECOMBINASE IN MICE

* * *

August 14, 2000

BRIEF UNDER 37 CFR § 1.191 ET SEQ.

Hon. Commissioner for Patents
Washington, D.C. 20231

Sir:

A Notice of appeal was timely filed pursuant to Rule 191(a) on March 14, 2000. This brief is now filed in triplicate to appeal the Examiner's final rejection of the pending claims. Reversal of that final rejection is respectfully requested.

A petition and fee for a three-month extension to the due date for this brief is being filed herewith. Please charge the total fee of $530 to our deposit account 03-3975 under order no. 20263/234805.

(1) Real Party in Interest

By assignment recorded at the U.S. Patent and Trademark Office on May 15, 1998 starting at reel 9179/frame 0078, rights in the subject invention were assigned to the Carnegie Institution of Washington.

(2) Related Appeals and Interferences

Appellant, her legal representatives, and the assignee are unaware of any related appeal or interference which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

08/15/2000 SLUANG1 00000062 033975 08866279
02 FC:217 380.00 CH

(3) Status of Claims

Claims 1-49 are pending and stand rejected.

08/15/2000 SLUANG1 00000062 033975 08866279
01 FC:220 150.00 CH

(4) Status of Amendments

The Examiner made the rejection of claims 1-49 final in the Office Action mailed September 14, 1999. Claim amendments were proposed subsequent to that final rejection by Appellant on March 14, 2000. The Examiner indicated that those proposed amendments would be entered and have overcome the rejection of claims 15, 42-43 and 47 under 35 U.S.C. 112, second paragraph.

The claims on appeal are set forth in the Appendix.

(5) Summary of Invention

The claimed invention is directed to transgenic mice having sufficient Flp recombinase activity such that recombination between Flp-recognition sequences is catalyzed, methods of *in vivo* genetic engineering using such transgenic mice, and systems thereof for genetic manipulation. *See* pages 3-9 of the specification and the original claims. Thus, the invention as presently claimed is fully supported by Appellant's original disclosure.

(6) Issues

A. Under 35 U.S.C. 102(b) was it proper to reject claims 1-2, 4-19, 22-27, 29-36, 41-43, 45 and 48 as allegedly being anticipated by Kilby et al. (1993)?

B. Under 35 U.S.C. 102(b) was it proper to reject claims 1-2, 4-13, 22-27, 29-33, 41-43, 45 and 48 as allegedly being anticipated by Wigley et al. (1994)?

C. Under 35 U.S.C. 103(a) was it proper to reject claims 1-2, 4-13, 15, 22-27, 29-33, 37-43, 45 and 47-48 as allegedly being unpatentable over Lakso et al. (1992), Wigley et al. (1994), Marx (1993), Marshall (1989), and Bieche et al. (1992)?

D. Under 35 U.S.C. 103(a) was it proper to reject claims 3, 21, 28, 44, 46 and 49 as allegedly being unpatentable over Wigley et al. (1994), Panigrahi et al. (1992), O'Gorman et al. (1991), Wahl et al. (1997), Hartley et al. (1980), and Buchholz et al. (1996)?

E. Under 35 U.S.C. 103(a) was it proper to reject claims 1, 12, 15, 20, 24, 43 and 47 as allegedly being unpatentable over Orban et al. (1992) and Wigley et al. (1994)?

Appellant submits that the Examiner's final rejections are improper for the reasons discussed below and respectfully request their reversal by the Board of Patent Appeals and Interferences (i.e., the "Board").

(7) Grouping of Claims

Claims 1-49 stand or fall together.

(8) Arguments

A. Kilby et al. and Wigley et al. Do Not Place a Transgenic Mouse With Sufficient Flp Recombinase Activity Such That Recombination Between Flp-Recognition Sequences Is Catalyzed In the Possession of the Public

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Similarly, to establish *prima facie* obviousness of a claim, all the limitations of that claim must be taught or suggested by the prior art. M.P.E.P. § 2143.03 citing *In re Royka*, 180 USPQ 580, 583 (C.C.P.A. 1974).

Appellant's claims are directed to transgenic mice, methods of *in vivo* genetic engineering using such transgenic mice, and systems for genetic manipulation in which the transgenic mouse has sufficient Flp recombinase activity such that recombination between Flp-recognition sequences is catalyzed.

On page 5 of Paper No. 4, the Examiner noted that "Kilby et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences." Table 1 of Kilby et al. shows that Flp-mediated recombination in transgenic mice had not been accomplished their knowledge, even though Flp appears to have been used in more different species than Cre. In the absence of a showing that Kilby et al. teach each and every element of the claimed invention, a *prima facie* case of anticipation has not been made.

In response to this argument, the Examiner stated on page 5 of Paper No. 14 that "Kilby et al. suggests making exactly the transgenic animals instantly claimed" (emphasis added). But this statement does not show that the public was put in possession of the claimed invention because, although the art may have recognized the desirability of making Appellant's invention, there is an absence of evidence that

the prior art established a reasonable expectation of success. At best, this assertion about Kilby et al. provides a motivation to make the claimed invention, but this is an insufficient basis for a rejection under Section 102.

The same argument was made with respect to Wigley et al. On page 7 of Paper No. 4, the Examiner noted that "Wigley et al. did not reduce to practice the generation and use of transgenic mice with the FLP recombinase gene and FRT target sequences." In the absence of a showing that Wigley et al. teach each and every element of the claimed invention, a *prima facie* case of anticipation has not been made.

In response, the Examiner stated on page 5 of Paper No. 14 that "Wigley et al. suggest making exactly the transgenic mice instantly claimed" (emphasis added). But this statement does not show that the public was put in possession of the claimed invention because, although the art may have recognized the desirability of making Appellant's invention, there is an absence of evidence that the prior art established a reasonable expectation of success. At best, this assertion about Wigley et al. provides a motivation to make the claimed invention, but this is an insufficient basis for a rejection under Section 102.

Finally, declaration evidence indicates that Appellant was the first person to make the claimed invention. See ¶8 of Dr. Robert Hammer's Declaration submitted on March 14, 2000. This was not contradicted by any evidence presented by the Examiner showing that either Kilby et al. or Wigley et al., or any other person, has made a transgenic mouse with functional Flp recombinase before the priority date of this application.

Therefore, because Kilby et al. and Wigley et al. do not teach "each and every element" of the claimed transgenic mouse, these references do not anticipate the claims and Appellant respectfully requests that the Section 102(b) rejections should be reversed by the Board.

B. Prior Art Does Not Show a Reasonable Expectation of Success and Evidence of Failed Attempts To Make the Claimed Invention Refute the Examiner's Conclusion of Unpatentability

Evidence that there was some predictability of success in combining elements of the cited references as proposed by the Examiner (i.e., a reasonable expectation of success) is required to support a conclusion of *prima facie* obviousness. *See In*

re Rinehart, 189 USPQ 143, 148-149 (C.C.P.A. 1976). Even if *prima facie* obviousness has been established, when evidence is submitted in rebuttal (e.g., secondary considerations like the failure of others), patentability must be reconsidered in the light of all evidence. Id. at 147; *Graham v. John Deere*, 148 USPQ 459, 467 (1966).

The cited references teach, at best, the desirability of Appellant's invention but they do not provide evidence of a reasonable expectation of success or how to arrive at the claimed invention when the evidence of record shows that making a transgenic mouse having sufficient Flp recombinase activity such that recombination between Flp-recognition sequences is catalyzed was not obvious.

First, on page 9 of Paper No. 4, the Examiner alleged there was a reasonable expectation of success because "the Cre-lox system had already been successfully employed to activate an oncogene in a transgenic mouse." It was further alleged that "the FLP recombinase system is analogous to the Cre recombinase system and functions in a manner that is mechanistically identical to the activity of Cre." Results with Cre cannot be so easily analogized to Flp, however, because the two recombinases do not appear to be identical in their enzymatic functions: (1) Gu et al. (1993), submitted on May 20, 1998, taught Flp recombinase was not as efficient as Cre recombinase in catalyzing recombination in ES cells (page 1160); (2) Sauer (1994), submitted on January 14, 1999, stated that Flp catalyzes excision less efficiently than Cre in ES cells (page 524); (3) Barinaga (1994), submitted on May 20, 1998, reported that Flp recombinase got a bad reputation when several groups tried to use it to make knockout mice because they had trouble getting it to work well in ES cells (page 28). In contrast, Appellant's specification discloses that Flp recombinase expressed according to the invention can achieve efficient recombination in ES cells on an extrachromosomal substrate (page 43).

The foregoing evidence shows that there was not a reasonable expectation of success before the present invention was made, there was a long lapse of time (about four years) between the publications disclosing transgenic mice with Cre and then Flp, Appellant was the first to put the public in possession of the claimed invention, and only one other Flp transgenic line has been published more than two and one-half years after Appellant's initial publication.

Second, on page 11 of Paper No. 4, the Examiner alleged that a reasonable expectation of success would have been anticipated because "the FLP recombinase

gene and FRT target sequences had already been used successfully in cultured mammalian cells as well as in transgenic *Drosophila* (as described in the discussion of the Kilby et al. reference)." Appellant submits that results in tissue culture or with transgenic *Drosophila* cannot be so easily extrapolated to use of Flp in transgenic mice. The recombinase activities documented in Table 1 of Kilby et al. suggests asking the question of why Flp transgenic mice were not done if these results could be readily applied in another context (i.e., a transgenic mouse).

The determination of the thermostability of Flp and Cre recombinases by Buchholz et al. suggests a possible answer to this question and an explanation for the failures of others to make the claimed invention prior to Appellant's success: a much lower temperature optimum for Flp than Cre. The abstract of Buchholz et al. (1996) states, "FLP is more thermolabile, having an optimum near 30°C and little detectable activity above 39°C . . . . Cre is optimally efficient at 37°C and above." They go on to disclose that the F70L mutation in a commercially available plasmid containing the FLP gene renders the Flp recombinase even more thermolabile. Buchholz et al. recommend "the use of Cre for applications in mice that require efficient recombination." More recent work by Buchholz et al. (1998), submitted on January 14, 1999, stated their goal was to obtain "an improved FLP recombinase that would redress inactivation by temperatures relevant to mammalian systems," in contrast to temperatures relevant to yeast (30°C) and *Drosophila* (25°C) systems in which Flp had been used (page 657). Caution is apparently needed if one assumes that recombinase activity in different cellular contexts will be identical because the improved Flp recombinase disclosed by Buchholz et al. (1998) is three- to five-fold better in cultured mammalian cells while it is four- to ten-fold better in *E. coli*.

Third, on page 13 of Paper No. 4, the Examiner alleged, "One would have anticipated a reasonable expectation of success because the analogous Cre-loxP system had already been successfully employed." As discussed above, however, the successful use of the Cre recombinase system in transgenic mice and the existence of the Flp-FRT system only establishes the long-felt need for Appellant's invention. But the evidence discussed above shows there was not a reasonable expectation of success when the present invention was made because of the different levels of recombinase activity for Cre and Flp.

The Examiner was not persuaded by the foregoing arguments.

On page 6 of Paper No. 14, the Examiner stated that the approach of Wigley et al. "would necessarily result in transgenic mice expressing FLP recombinase" (emphasis added). Appellant disagrees that such expression would necessarily occur in an amount sufficient to catalyze recombination between Flp-recognition sites. No evidence of record shows there was a reasonable expectation of success that mere integration of a Flp recombinase construct into the genome would result in its expression leading to sufficient Flp recombinase activity.

With respect to the Examiner's observation on page 7 of Paper No. 14 that O'Gorman asserted that Flp works nicely in transgenic mice, Appellant notes that the evidence is mixed. O'Gorman's assertion is not corroborated by any publication that Appellant is aware. When there are contradictory facts in evidence, they must be weighed against each other to determine whether there was a reasonable expectation of success. Cf. M.P.E.P. § 2143.01 ("where the teachings of the prior art conflict, the Examiner must weight the suggestive power of each reference"). When the negative published reports of several groups working with ES cells is weighed against the uncorroborated report of a single investigator (i.e., O'Gorman), it should be apparent the prior art did not show there was a reasonable expectation that a sufficient level of Flp recombinase activity could be achieved in transgenic mice.

On page 10 of Paper No. 14, Appellant was invited to submit evidence of the failed attempts of others to produce the claimed invention. This was done on March 14, 2000 when the Declaration of Dr. Robert Hammer was submitted.

Dr. Hammer performed the experiments described in his Declaration while a postdoctoral associate in Ralph Brinster's laboratory. Drs. Hammer and Brinster are widely recognized as experts in the production of transgenic mice and their use.

The experiments most relevant to the claimed invention are described in the Declaration. Briefly, a first transgenic mouse was made with tandemly integrated constructs, each containing a Flp-recognition sequence. This transgenic line was then used to produce fertilized eggs and they were injected with a Flp recombinase construct to make second transgenic mice. No recombination of Flp-recognition sequences was detected in the second transgenic mice.

In other experiments where it was attempted to provide Flp recombinase activity by injecting enzyme into fertilized eggs or transiently expressing an injected

Flp gene in fertilized eggs, no recombination of Flp-recognition sequences was detected.

This evidence (i.e., the failed attempts of others) is relevant to secondary considerations of nonobviousness and clearly shows that Appellant's invention is patentable. Such evidence went unrebutted by the Examiner who questioned in her Advisory Actions (Paper Nos. 17 and 20) whether the recombined gene (i.e., the product of the recombination) was functional. This is irrelevant to patentability under Section 103 because the usefulness and operability of the claimed invention (i.e., issues under Sections 101 and 112) are unchallenged by the rejections of record.

The Examiner appears to be asserting that activation of gene expression by recombination is essential for operability of the claimed invention. But as explained in Appellant's specification and the response filed on June 6, 2000, this is not the only use of the claimed invention because tracing cell lineages and deletion of sequences flanked by Flp-recognition sequences do not require transcription and translation of the product of recombination.

The Examiner's statements in Paper Nos. 17 and 20 do not rebut the declaration evidence that others have failed to make the claimed invention.

For the reasons discussed above, Appellant respectfully requests that the Section 103(a) rejections should be reversed by the Board.

Appellant submits that the pending claims are in condition for allowance and earnestly request an early Notice to that effect. The Board is invited to contact the undersigned if further information is needed.

Respectfully submitted,

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1. A transgenic mouse comprising a Flp transgene integrated in a genome of the transgenic mouse, wherein the Flp transgene is expressed in a cell of the transgenic mouse at a level of recombinase activity sufficient to catalyze recombination between Flp-recognition sequences of the cell.

2. The transgenic mouse according to Claim 1, wherein the genome further comprises a Flp-recognition sequence.

3. The transgenic mouse according to Claim 2, wherein the Flp-recognition sequence is SEQ ID NO:14 or SEQ ID NO:15.

4. The transgenic mouse according to Claim 2, wherein the transgenic mouse contains at least two diploid cells with different numbers of Flp-recognition sequences.

5. The transgenic mouse according to Claim 2, wherein the genome is hemizygous for the Flp-recognition sequence.

6. The transgenic mouse according to Claim 1, wherein the genome comprises at least two Flp-recognition sequences.

7. The transgenic mouse according to Claim 6, wherein the genome comprises at least two chromosomes, each chromosome comprising a Flp-recognition sequence.

8. The transgenic mouse according to Claim 1, wherein the genome further comprises two Flp-recognition sequences in direct repeat orientation.

9. The transgenic mouse according to Claim 1, wherein the genome further comprises two Flp-recognition sequences in inverted repeat orientation.

10. The transgenic mouse according to Claim 1, wherein the genome further comprises a Cre transgene.

11. The transgenic mouse according to Claim 1, wherein the genome further comprises a drug selectable marker transgene flanked by Flp-recognition sequences, wherein the drug selectable marker is excised in cells containing sufficient Flp recombinase activity.

12. The transgenic mouse according to Claim 1, wherein the genome further comprises another transgene flanked by Flp-recognition sequences.

13. The transgenic mouse according to Claim 12, wherein said another transgene is flanked by Flp-recognition sequences in direct repeat orientation.

14. The transgenic mouse according to Claim 12, wherein said another transgene is flanked by Flp-recognition sequences in inverted repeat orientation.

15. The transgenic mouse according to Claim 12, wherein said another transgene is selected from the group consisting of genes controlling differentiation of a cell or development of an organism, genes required for viability of a cell or organism, cytokine genes, neurotransmitter or neurotransmitter receptor genes, oncogenes, tumor suppressor genes, selectable markers, and histochemical markers.

16. The transgenic mouse according to Claim 15, wherein said another transgene is flanked by Flp-recognition sequences in direct repeat orientation.

17. The transgenic mouse according to Claim 15, wherein said another transgene is flanked by Flp-recognition sequences in inverted repeat orientation.

18. The transgenic mouse according to Claim 12, wherein expression of said additional transgene is activated in cells containing sufficient Flp recombinase activity.

19. The transgenic mouse according to Claim 12, wherein expression of said additional transgene is inactivated in cells containing sufficient Flp recombinase activity.

20. The transgenic mouse according to Claim 1, wherein Flp recombinase activity is regulated by a factor selected from the group consisting of chemical, developmental stage, temperature, and tissue type.

21. The transgenic mouse according to Claim 1, wherein the Flp transgene encodes amino acid sequence SEQ ID NO:17 or SEQ ID NO:19.

22. A transgenic mouse comprising a Flp transgene, wherein the Flp transgene is expressed in a cell of the transgenic mouse at a level of recombinase activity sufficient to catalyze recombination between Flp-recognition sequences of the cell.

23. A transgenic mouse comprising a genome which contains a Flp transgene and a Flp-recognition sequence, wherein the Flp-recognition site has undergone Flp-catalyzed recombination.

24. A method of *in vivo* genetic engineering comprising:

(a) providing a transgenic mouse comprising a genome which contains a Flp transgene and at least two Flp-recognition sequences,

(b) expressing the Flp transgene at a level of recombinase activity sufficient to catalyze site-specific recombination in a cell, and

(c) catalyzing recombination between the two Flp-recognition sequences of the cell.

25. The method according to claim 24, wherein site-specific recombination occurs in a germ line cell.

26. The method according to claim 25, further comprising:

(d) mating the transgenic mouse to produce an offspring comprising a recombined genome which does not contain the Flp transgene.

27. The method according to claim 24, wherein site-specific recombination occurs in a somatic cell.

28. The method according to Claim 24, wherein at least one of the Flp-recognition sequences is SEQ ID NO:14 or SEQ ID NO:15.

29. The method according to Claim 24, wherein the genome comprises at least two chromosomes and each chromosome contains a Flp-recognition sequence, whereby recombination between the two Flp-recognition sequences causes chromosomal translocation.

30. The method according to Claim 24, wherein the genome comprises a chromosome and the two Flp-recognition sequences are direct repeats flanking a target sequence on the chromosome, whereby recombination between the two Flp-recognition sequences causes excision of the target sequence.

31. The method according to Claim 30, wherein the target sequence is a drug selectable marker.

32. The method according to Claim 24, wherein the genome comprises a chromosome containing a first Flp-recognition sequence and a target sequence containing a second Flp-recognition sequence, whereby recombination between the two Flp-recognition sequences causes insertion of the target sequence into the chromosome.

33. The method according to Claim 24, wherein the genome comprises a chromosome containing a first Flp-recognition sequence and a plasmid containing a transgene and a second Flp-recognition sequence, whereby recombination between the two Flp-recognition sequences causes insertion of the transgene into the chromosome.

34. The method according to Claim 24, wherein the genome comprises a chromosome and the two Flp-recognition sequences are inverted repeats flanking a target sequence on the chromosome, whereby recombination between the two Flp-recognition sequences causes inversion of the target sequence.

35. The method according to Claim 34, wherein expression of the target sequence is increased by the inversion.

36. The method according to Claim 34, wherein expression of the target sequence is decreased by the inversion.

37. The method according to Claim 24, wherein recombination causes activation of an oncogene or inactivation of a tumor suppressor gene in the cell, thereby transforming the cell and establishing a probability of developing cancer in the transgenic mouse.

38. The method according to Claim 37, further comprising:

(d) administering a candidate agent to the transgenic mouse; and

(e) identifying the candidate agent as a cancer promoter if the probability of developing cancer increases or a cancer inhibitor if the probability of developing cancer decreases.

39. The method according to claim 37, wherein the oncogene is selected from the group consisting of ABL1, BCL1, BCL2, BCL6, CBFA2, CBL, CSF1R, ERBA, ERBB, EBRB2, ETS1, ETV6, FGR, FOS, FYN, HCR, HRAS, JUN, KRAS, LCK, LYN, MDM2, MLL, MYB, MYC, MYCL1, MYCN, NRAS, PIM1, PML, RET, SRC, TAL1, TCL3, and YES.

40. The method according to claim 37, wherein the tumor suppressor gene is selected from the group consisting of APC, BRCA1, BRCA2, DCC, MADH4, MCC, NF1, NF2, RB1, WT1, and TP53.

41. The method according to claim 24, wherein Flp-mediated recombination activates ectopic expression of a gene controlling differentiation of a cell or development of an organism.

42. The method according to claim 24, wherein Flp-mediated recombination inactivates post-embryonic expression of a gene controlling embryonic development of the transgenic mouse.

43. The method according to claim 24, wherein Flp-mediated recombination identifies a cell lineage in the transgenic mouse.

44. The transgenic mouse according to Claim 24, wherein the Flp transgene encodes amino acid sequence SEQ ID NO:17 or SEQ ID NO:19.

45. A system for genetic manipulation, comprising:

(a) the transgenic mouse according to Claim 22, and

(b) a purified nucleic acid comprising a Flp-recognition sequence.

46. The system according to Claim 45, wherein the Flp-recognition sequence is SEQ ID NO:14 or SEQ ID NO:15.

47. The system of claim 45, wherein the purified nucleic acid further comprises a sequence selected from the group consisting of genes controlling differentiation of a cell or development of an organism, genes required for viability of a cell or organism, cytokine genes, neurotransmitter or neurotransmitter receptor genes, oncogenes, tumor suppressor genes, selectable markers, and histochemical markers.

48. The system of claim 45, further comprising:

(c) means for producing a transgenic mouse comprising a genome which contains the Flp-recognition sequence.

49. The system of claim 45, wherein the Flp transgene encodes amino acid sequence SEQ ID NO:17 or SEQ ID NO:19.